

***Remarks***

Reconsideration of this Application is respectfully requested.

Claims 1, 3-10, 13, 16, 17, 19, 26, 28, 29 and 34-38 are pending in the application.

All the pending claims stand rejected.

Based on the following remarks, Applicant respectfully requests that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

***Information Disclosure Statement***

Applicant notes that the Examiner did not initial document AP1 cited in the First Supplemental Information Disclosure Statement filed on August 28, 2000. (See copy of Information Disclosure Statement attached to Paper No. 12.) Applicant respectfully requests that the Examiner initial the document cited in the Information Disclosure Statement or indicate why it was not considered.

In addition, Applicant wishes to clarify the certification made in the Information Disclosure Statement filed on November 21, 2001. In the certification, Applicant stated that "no item of information in the Information Disclosure Statement was cited in a communication from a foreign patent office in a counterpart foreign application and, to my knowledge after making reasonable inquiry, was known to any individual designated in 37 C.F.R. § 1.56(c) more than three months prior to the filing of this Information Disclosure Statement. 37 C.F.R. § 1.97(e)(2)." This certification is correct since the cited patent issued less than three months prior to the filing of this Information Disclosure Statement.

However, Applicant was aware of the patent application that ultimately issued as the patent more than three months prior to the filing of the IDS. If the Examiner requires any additional information regarding this certification, he is invited to contact the undersigned.

***Rejections under 35 U.S.C. § 112***

The Examiner maintained the rejection of claims 1, 3-10, 13, 16-17, 19, 26, 28-29 and 34-44 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. (Paper No. 15, at page 2.) The Examiner alleged that

[t]he claimed genus includes all possible *Thermotoga maritima* (*Tma*) DNA polymerase mutants which are modified at least two ways selected from the group consisting of (a) to reduce or eliminate the 3' 5' exonuclease acitivity of the polymerase; (b) to reduce or eliminate the 5' 3' exonuclease activity of the polymerase; and (c) to reduce or eliminate discriminatory behavior against a dideoxynucleotides and methods of using and kits comprising said DNA polymerase mutants and genes encoding said DNA polymerase mutants. As stated in the previous office aciton [sic], there is no disclosure of any particular structure to function/activity relationship in the claimed genus. While the specification provides the species, Asp<sup>323</sup>→Ala<sup>323</sup>, (having reduced 3'→5' exonuclease activity), Phe<sup>730</sup>→Tyr<sup>730</sup> (having reduced discriminatory behavior against a dideoxynucleotides) and Asp<sup>8</sup>→Ala<sup>8</sup>, Asp<sup>137</sup>→Ala<sup>137</sup> or the deletion of 219 amino terminal amino acids of Tne [sic, *Tma*] DNA Polymerase (having reduced 5'→3' exonuclease activity) encompassed by these claims, the specification clearly does not disclose a representative number of species of the claimed genus which includes an infinite number of amino acid variants as well as additional chemical modifications of any *Tma* DNA polymerases. Even considering the substantial knowledge of the skilled artisan, as detailed by applicants, one could only envision a small number of additional species within the scope of the claimed

genus. However, in view of the enormous breath of the claimed genus, even these could in no way be considered to be representative of the entire genus. There is no disclosure of any particular structure to function/activity relationship in the claimed genus. The claimed genus of mutant *Tma* DNA polymerases has **no** structural limitation, only a functional limitation, thus the infinite number of species encompassed by this genus has not been adequately described by the few species disclosed in the specification.

*Id.* at pages 3-4 (emphasis in original).

Applicant respectfully disagrees with the Examiner and submits that one skilled in the art could reasonably conclude that Applicant had possession of the *Tma* DNA polymerase mutants encompassed by the rejected claims, in the present application as filed.

The test for the written description requirement is whether one skilled in the art could reasonably conclude that the inventor has possession of the claimed invention in the specification as filed. *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563, 19 U.S.P.Q.2d 1111, 1116 (Fed. Cir. 1991); M.P.E.P. § 2163.02. The Examiner bears the initial burden of presenting a *prima facie* case of unpatentability. This burden is discharged if the Examiner can present evidence or reasons why one skilled in the art would not reasonably conclude that Applicant possessed the subject matter as of the priority date of the present application.

*In re Wertheim*, 541 F.2d 257, 262, 191 USPQ2d 90, 96 (C.C.P.A. 1976); M.P.E.P. § 2163.04. In the instant case, the Examiner has not met this burden.

Moreover, the Guidelines for Examination of Patent Applications Under the 35 U.S.C. § 112, paragraph 1, "Written Description" Requirement state that

[t]he analysis of whether the specification complies with the written description requirement calls for the examiner to compare the scope of the claim with the scope of the description to determine whether applicant has demonstrated possession of the claimed invention. Such a review is conducted from the standpoint of one of skill in the art at the

time the application was filed and should include a determination of the field of the invention and the level of skill and knowledge in the art. *Generally, there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosure necessary to satisfy the written description requirement.*

66 Fed. Reg. 1099, 1105 (Jan. 5, 2001)(emphasis added). More specifically, regarding claims drawn to a genus, the Guidelines state that

a "representative number of species" means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. *On the other hand, there may be situations where one species adequately supports a genus. What constitutes a "representative number" is an inverse function of the skill and knowledge in the art. Satisfactory disclosure of a "representative number" depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed.* For inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus. *Description of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces.*

66 Fed. Reg. 1099, 1106 (Jan. 5, 2001)(emphases added).

Applicant submits that the Examiner has underestimated both the level of skill and knowledge in the art on the priority date of the present application and the teaching of the present application. The level of skill and knowledge in the art on the priority date of the present application was substantial. At the time the invention was made, the sequence of many DNA polymerases had been compiled and aligned. These DNA polymerases can be divided into several distinct families based on sequence homology. Family A, or Pol I-type

polymerases, exhibit significant homology to *E. coli* DNA polymerase I. Family B, or Pol II-type polymerases, exhibit significant homology to *E. coli* DNA polymerase II. Family C, or Pol III-type polymerases, exhibit significant homology to *E. coli* DNA polymerase III alpha subunit. One skilled in the art would easily be able to determine that *Tma* DNA polymerase exhibits significant homology to *E. coli* DNA polymerase I and is, thus, part of the Pol I-type family of polymerases.

In addition, the specification teaches that the *Thermotoga* DNA polymerase of the invention can be isolated from any strain of *Thermotoga* which produces a DNA polymerase. Thus, although the preferred strain to isolate the gene encoding the *Thermotoga* DNA polymerase of the present invention is *Thermotoga maritima* (*Tma*), Applicant asserts that additional DNA polymerases, including allelic variants, can be isolated from other *Thermotoga* strains. See specification at page 16, lines 13-24. This is easily accomplished using standard molecular biology protocols since all DNA polymerases contain several conserved regions, including the regions that contain, *inter alia*, 3' to 5' exonuclease activity and 5' to 3' exonuclease activity. See specification at page 18, line 26, to page 19, line 25. Mutating specific amino acid residues within these regions abolishes or reduces these activities. *Id.* Examples of other DNA polymerases that can be so mutated are indicated on page 20, lines 8-13, of the specification.

Furthermore, by comparison of the amino acid sequence of *Tma* DNA polymerase to other DNA polymerases, consensus sequences can be identified. For example, the O-helix is a 14 amino acid sequence that when mutated renders the polymerase non-discriminating against non-natural nucleotides, such as dideoxynucleotides. See specification at page 21, line 21, to page 22, line 14. The consensus sequence of the O-helix

may be defined as **RXXXKXXXFXXXXX**, wherein X is any amino acid. *Id.* A review of the *Tma* DNA polymerase amino acid sequence, as provided in U.S. Patent No. 5,374,553 (which is incorporated by reference as indicated at page 18 of the specification), shows significant homology to the O-helix consensus sequence: amino acids 722-735 **RRAGKVNFSIIYG**. Additional consensus sequences common among DNA polymerases are described below.

The preceding discussion illustrates that one skilled in the art looking at the amino acid sequence of *Tma* DNA polymerase and comparing it to other known DNA polymerases, can easily locate conserved regions and identify consensus sequences or motifs. Knowledge of these conserved regions or motifs would allow one skilled in the art of molecular biology to identify regions that can be mutated. For example, the specification discloses at page 19, lines 4-10, that the 3'-5' exonuclease activity can be reduced or impaired by creating site specific mutants within the 3'-5' exonuclease domain. In a specific embodiment of the invention Asp<sup>323</sup> of *Tma* may be changed to any other amino acid, preferably to Ala to substantially reduce 3'-to-5' exonuclease activity.

The specification further discloses that the 5'-3' exonuclease activity of the *Tma* DNA polymerase can be reduced or eliminated by mutating the DNA polymerase gene. Such mutations include point mutations, frame shift mutations, deletions, and insertions. For example, any one of six conserved amino acids that are associated with the 5'-3' exonuclease activity can be mutated. Examples of these conserved amino acids with respect to *Tma* DNA polymerase include Asp<sup>8</sup>, Glu<sup>112</sup>, Asp<sup>114</sup>, Asp<sup>115</sup>, Asp<sup>137</sup> and Asp<sup>139</sup>. See specification at page 19, lines 11-25.

*Tma* DNA polymerase mutants can also be made to render the polymerase non-discriminating against non-natural nucleotides such as dideoxynucleotides. See specification at pages 21-22. Changes within the O-helix of *Tma* polymerase, such as other point mutations, deletions, and insertions, can be made to render the polymerase non-discriminating. *Id.* The most important amino acids in conferring discriminatory activity include Arg, Lys and Phe. Amino acids which may be substituted for Arg at positions 722 are selected independently from Asp, Glu, Ala, Val Leu, Ile, Pro, Met, Phe, Trp, Gly, Ser, Thr, Cys, Tyr, Gln, Asn, Lys and His. Amino acids that may be substituted for Phe at position 730 include Lys, Arg, His, Asp, Glu, Ala, Val, Leu, Ile, Pro, Met, Trp, Gly, Ser, Thr, Cys, Tyr, Asn or Gln. Amino acids that may be substituted for Lys at position 726 include Tyr, Arg, His, Asp, Glu, Ala, Val, Leu, Ile, Pro, Met, Trp, Gly, Ser, Thr, Cys, Phe, Asn or Gln. Most preferred mutants include Phe<sup>730</sup> to Tyr<sup>730</sup>, Ser<sup>730</sup>, Thr<sup>730</sup> and Ala<sup>730</sup>. *Id.*

In addition, the specification discloses at pages 19 and 20 that to abolish 5'-3' exonuclease activity, amino acids are selected which have different properties. For example, an acidic amino acid such as Asp may be changed to a basic, neutral or polar but uncharged amino acid such as Lys, Arg, His (basic); Ala, Val, Leu, Ile, Pro, Met, Phe, Trp (neutral); or Gly, Ser, Thr, Cys, Tyr, Asn or Gln (polar but uncharged). Glu may be changed to Asp, Ala, Val Leu, Ile, Pro, Met, Phe, Trp, Gly, Ser, Thr, Cys, Tyr, Asn or Gln.

As evidence that the level of skill and knowledge in the art on the priority date of the present application was substantial, Applicant further discusses the various known common attributes and features of *Thermotoga* DNA polymerases below, and discusses various scientific documents to strengthen and support his arguments.

The *Tma* DNA polymerases are part of the Pol I-type family of polymerases and exhibit significant homology to *E. coli* DNA polymerase I. As discussed *supra*, all DNA polymerases contain conserved regions, each of which has a consensus sequence. For example, Pol I-type DNA polymerases share many conserved sites throughout the protein. Gutman *et al.*, *Nucl. Acids Res.* 21:4406-4407 (1993) (reference AR6), describe conserved sites in the 5'-3' exonuclease domain of Pol I-type DNA polymerases. Six conserved regions, identified as regions A-F, are shown in Figure 1 of Gutman *et al.* and a consensus sequence for each of these conserved regions is provided. A review of the *Tma* DNA polymerase amino acid sequence, as provided in U.S. Patent No. 5,374,553 (which is incorporated by reference as indicated at page 18 of the specification), shows significant homology to these consensus sequences.

Similarly, Bernard *et al.*, *Cell* 59:219-228 (1989) (reference AR2), describe conserved regions in the 3'-5' exonuclease domain of Pol I-type polymerases. There is significant similarity between these conserved regions and corresponding regions in the *Tma* DNA polymerase. Additional information regarding the domain structure of *Tma* DNA polymerases, including the localization of the exonuclease activities and polymerase activity to specific domains, is available. For example, the 5'-3' exonuclease domain of *E. coli* polymerase I is at the N-terminal end of the polymerase, while the middle and C-terminal domains of this polymerase exhibit 3'-5' exonuclease and DNA polymerase activity, respectively. See C. M. Joyce, *Curr. Opin. Str. Biol.* 1:123-129 (1991) (reference AS7). This domain structure is conserved in other Pol I-type polymerases, such as *Tma* DNA polymerase.

Further information regarding the domain structure of the *Tma* DNA polymerase can be seen from the functional characterization of deletion mutants of *Tne* DNA polymerase and several other Pol I-type polymerases. For example, deletion of the first 235 and 288 amino acids from the N-terminal region of *Taq* polymerase eliminates 5'-3' exonuclease activity. Similarly, deletion of the first 250 amino acids of the *Tth* polymerase and the first 323 amino acids of *E. coli* polymerase I also eliminates 5'-3' exonuclease activity. Deletion of the first 514 and 520 amino acids from the N-terminal region of *E. coli* polymerase I eliminates both 5'-3' and 3'-5' exonuclease activity.

Thus, the sequence and other information disclosed for DNA polymerases in the specification would indicate to a scientist skilled in the art that polymerase activity is localized to the C-terminal portion of the *Tma* DNA polymerase, while the 5'-3' and 3'-5' exonuclease activities are localized to the N-terminal and middle portions of this polymerase, respectively. Fragments containing the C-terminal region will typically retain polymerase activity, while fragments lacking the 5'-3' or 3'-5' exonuclease domains will not retain 5'-3' or 3'-5' exonuclease activity, respectively.

In addition, significant additional information regarding mutant forms of *Tma* DNA polymerase with reduced or no 5'-3' or 3'-5' exonuclease activity, or reduced or no discriminatory behavior against a dideoxynucleotide can be obtained when coupled with structural information that has been obtained for *E. coli* polymerase I and other Pol I-type DNA polymerases. For example, a high resolution three-dimensional crystal structure for the Klenow fragment of *E. coli* polymerase I has been reported by Ollis *et al.*, *Nature*

313:762-766 (1985) (reference AS7).<sup>1</sup> In this structure, regions that may be associated with polymerase and 3'-5' exonuclease activity are identified. Based on the sequence homology between *E. coli* polymerase I and the *Tma* polymerase, the *Tma* polymerase and *E. coli* polymerase I are expected to have a similar structure. Thus, this additional structural information provides extensive guidance to one skilled in the art to select mutants having reduced or eliminated 3'-5' exonuclease activity, while retaining polymerase activity. Based on the sequence homology between *E. coli* polymerase I and the *Tma* DNA polymerase, a scientist skilled in the art would readily be able to identify segments or specific residues of the *Tma* DNA polymerase involved in polymerase and 3'-5' or 5'-3' exonuclease activity.

Further guidance to select fragments or mutants of *Tma* DNA polymerase would be provided to a scientist skilled in the art from mutational analyses performed on *Tne* DNA polymerase and other Pol I-type DNA polymerases. Polesky *et al.*, *J. Biol. Chem.* 265:14579-14591 (1990) (reference AR8) describe several mutations within the polymerase domain of the Klenow fragment of *E. coli* polymerase I. Mutations at residues 849, 668 and 882 show a large decrease in catalytic activity. Thus, several residues involved in polymerase activity have been identified. Each of these residues are within regions of high homology. Moreover, these residues are located near the region predicted to be associated with polymerase activity in the three-dimensional structure of the Klenow fragment. In a further article, several additional mutations are analyzed, confirming the location of the active site. See Polesky *et al.*, *J. Biol. Chem.* 267:8417-8428 (1992) (reference AT7).

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<sup>1</sup> The Klenow fragment of *E. coli* polymerase I also contains the 3'-5' exonuclease domain. Thus, this article also provides useful information regarding the 3'-5' exonuclease active site.

Mutational analyses have also been performed in the 3'-5' exonuclease domain of *E. coli* polymerase I. See Derbyshire *et al.*, *Science* 240:199-201 (1988) (reference AR3); and Derbyshire *et al.*, *EMBO J.* 10:17-24 (1990) (reference AT2). The mutations that resulted in loss of exonuclease activity, at residues 355, 424 and 501 were located in the regions of the 3'-5' exonuclease domain exhibiting a high degree of homology with *The* DNA polymerase and other members of the *E. coli* polymerase I family of polymerases. This information provides guidance to one skilled in the art to envision numerous specific mutations, in addition to those described in the specification, that will reduce or eliminate 3'-5' exonuclease activity.

Similarly, Pol I-type DNA polymerase mutants have been made containing mutations in the 5'-3' exonuclease domain. Several of these mutations, falling within regions of homology, reduce or eliminate 5'-3' exonuclease activity. Thus, these experiments also confirm that the selection of specific residues based on sequence homology can identify mutants expected to have reduced or eliminated 5'-3' exonuclease activity.

As discussed above, generally, there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosure necessary to satisfy the written description requirement. In this situation: 1) there is detailed and well known structural information for DNA polymerases; 2) structure/function correlations are available for these DNA polymerases; and 3) the sequence and other structural information for the *Tma* DNA polymerases are provided in this and the priority applications. Thus, the level of skill and knowledge in the art is substantial. Accordingly, the specificity of disclosure necessary to satisfy the written description requirement is minimal.

In summary, Applicant submits that: (1) the level of skill and knowledge of one skilled in the art at the time the application was filed was substantial; (2) adequate written description of a genus which embraces variant species can be achieved by disclosing only one species within the genus; (3) one skilled in the art would recognize that Applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed; (4) individual support for each species that the genus embraces is not required; and (5) based on the substantial level of skill and knowledge in the art, and the fact that Applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed, the disclosure in the specification necessary to satisfy the written description requirement is sufficient. Accordingly, withdrawal of the written description rejection under 35 U.S.C. § 112, first paragraph, is respectfully requested.

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***Conclusion***

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicant believes that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

CHATTERJEE, Deb K.  
Appl. No. 09/229,173

Prompt and favorable consideration of this Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

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